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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl cow feeding and metabolism studies submitted in response to a 3(c)(2)(b) letter.

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In response to 3(c)(2)(b) letters of 12/29/83 (D. Campt, RD, to J. Trexel, Dupont) and 5/14/85 (R. Brown, RD to J. Trexel, Dupont), Dupont has submitted two cow metabolism studies. For one study, cows were dosed with ^{14}C-benomyl and for the other study, cows were dosed with ^{14}C-carbendazim (MBC, methyl benzimidazole carbamate). Also submitted are two cow feeding studies; in one, benomyl was fed, and in the other MBC was fed.

The most recent 3(c)(2)(b) letter requires that Dupont "confirm the residue level values in milk and the plateau level of bound residues in liver." Dupont states that the studies now submitted are intended to address the question of an appropriate tolerance level for benomyl and its metabolites in milk. These studies are apparently not intended to address the question of a plateau level for bound residues in liver.

The submitted studies are summarized here:

"Metabolism of [2-^{14}C] Benomyl in the Lactating Dairy Cow"

A lactating Holstein dairy cow was dosed with [2-^{14}C]-benomyl at a level equivalent to 50 ppm in the diet for five days. Milk, urine, and feces were collected twice daily, and the cow was sacrificed about 17 hours after the final dose. A blood sample was taken, and the following tissues were removed for analysis: brain, heart, kidney, liver, mammary gland, pancreas, omental fat, renal fat, subcutaneous fat, skeletal muscle, and spleen.

Tissue samples were combusted, and the resulting $^{14}CO_2$ was determined by liquid scintillation counting (LSC). Results are summarized in the following table:

Tissue	ppm in Benomyl equivalents
muscle	0.02
liver	4.12
fat	0.01-0.04
kidney	0.25
milk	0.16-0.23

Activity in milk, liver, and kidney was characterized as follows.

Milk

A 100 g sample of milk was refluxed with 10 mL of 85% phosphoric acid for one hour. The milk was cooled, then extracted with n-hexane. The hexane fraction contained less than 0.01% of the milk activity.

The pH was adjusted to 6.5 with 50% NaOH, and the milk solution was then extracted with ethyl acetate. About 50% of the activity was soluble in ethyl acetate, 36% was soluble in water, and 8% was isolated as solids. The ethyl acetate layer was comprised mainly of methyl (5-hydroxy-1H-benzimidazol-2-yl)carbamate (5-HBC; 29% of the total activity) and methyl (4-hydroxy-1H-benzimidazol2-yl)-carbamate (4-HBC; 16%). Sixty-two percent of the activity in the aqueous phase (22% of the total activity) corresponded by HPLC to methyl (4,5-dihydro-4,5-dihydroxy-1H-benzimidazol-2-yl)carbamate, which Dupont has dubbed metabolite A. This metabolite was also found in urine and had been identified by mass spectroscopy. Treatment of the aqueous phase with HCl produced a mixture of 4-HBC and 5-HBC, dehydration products of metabolite A, in low yield.

Of the total milk activity, 16% was identified as 4-HBC, 29% as 5-HBC, and 22% as metabolite A. The solids contained 8% of the activity, and this wasn't further characterized. Of the organo-soluble phase, 4% wasn't identified, and of the aqueous phase, 14% wasn't identified, though the registrant speculates that this is also metabolite A.

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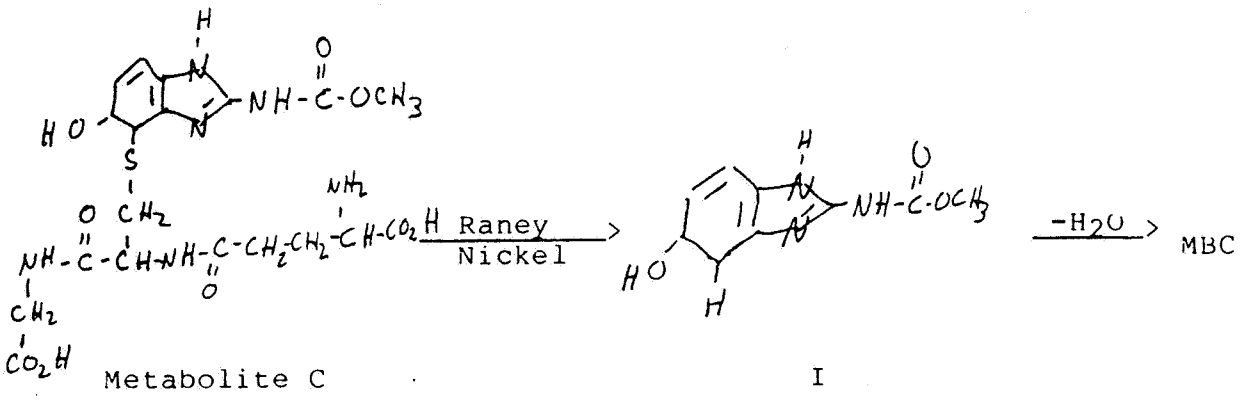
Liver

A liver sample was blended with 15N phosphoric acid, then heated in a steam bath for one hour. The sample was cooled, the pH was adjusted to 6.5, and it was then extracted with ethyl acetate. The ethyl acetate contained 5% of the total liver activity; this material was identified as 5-HBC.

A second liver sample was treated with Raney nickel, then hydrolyzed and extracted as described above. This resulted in 11% of the total liver activity being soluble in ethyl acetate. Two-thirds of this was identified as MBC.

Based on studies in the open literature, the registrant speculates that Raney nickel desulfurizes conjugates such as metabolite C (shown below) to form I, which readily dehydrates in the presence of acid to form MBC.



Metabolite C I

No other liver activity was characterized.

The petitioner points out that these studies are consistent with an earlier goat metabolism study ("Attempts to Characterize Liver Residues from ^{14}C-Benomyl Dosed Goat," P.T. Hardesty, Dupont, submitted with PP#6F1748). In that study most of the liver activity was bound, and treatment with protease enzymes released it into a water-soluble form. Chromatography resolved this material into many components, none of which were identified.

Kidney

A kidney sample was treated with phosphoric acid, then extracted with ethyl acetate. The organic layer contained 52% of the kidney activity, 49% as 5-HBC, and 3% as 4-HBC. The aqueous layer contained 21%, and the remainder wasn't extracted. Metabolite A was found in the aqueous layer and comprised 4% of the total activity.

"Metabolism of [2-^{14}C]-Carbendazim in the Lactating Dairy Cow"

A lactating Holstein was dosed twice daily for five days with [2-^{14}C]-carbendazim (MBC) at a rate equivalent to 50 ppm in the diet.

Samples of urine, feces, and milk were collected at each dose, and the animal was slaughtered seventeen hours after the final dose. Organs, tissues, and blood samples were taken for analysis.

Activity was quantified by liquid scintillation counting of $^{14}CO_2$ from combusted samples. Results are tabulated below:

Tissue	ppm in MBC equivalents
muscle	0.01
liver	2.26
kidney	0.45
fat	0.01-0.09
milk	0.22-0.29

Activity in milk, urine, liver, and kidney was characterized as follows:

Milk

A 100 g sample of milk was refluxed with 10 mL of 85% phosphoric acid for one hour. This solution was cooled, then extracted with n-hexane, which took up <0.1% of the activity.

The milk was brought to pH=6.5 with 50% NaOH, and then extracted three times with 100 mL portions of ethyl acetate. Seventy percent of the activity dissolved in the ethyl acetate, 25% remained in the aqueous phase, and 3% was isolated in solids.

The ethyl acetate phase was washed with 0.1M HCl to remove MBC and its metabolites from acidic lipids. The ethyl acetate now contained <5% of the total activity; it was discarded. The aqueous phase was again brought to pH=6.5 with 50% NaOH, and was then extrated with ethylacetate. The concentrated residue was subjected to TLC to identify metabolites; quantitaion was by a linear analyzer. The most significant metabolites uncovered in the ethyl acetate phase were 5-HBC (42% of the total milk activity) and 4-HBC (21%).

Sixty-five percent of the aqueous activity (i.e., about 16% of the total milk activity) was found by HPLC to be metabolite A (carbendazim dihydrodiol). Treatment of this material with HCl produced some 5-HBC and 4-HBC.

Liver

Hydrolysis with phosphoric acid followed by ethyl acetate extraction released only 1% of the liver activity. This material was identified as 5-HBC. Treatment of the liver tissue with Raney nickel prior to hydrolysis and ethyl acetate extraction released 19% of the activity. Most of this activity (80%; 15% of the total activity) was identified as MBC.

"Residue Study of the Fungicide MBC in Lactating Dairy Cattle" Document No. AMR-429-85

Twelve Holstein dairy cows, each producing 10Kg or more of milk daily, were dosed daily with MBC at levels of 2, 10, and 50 ppm in the diet, three animals at each level, for 28 days. Two of the animals in each group were sacrificed on day 29 and the third animal was sacrificed on day 36, seven days after cessation of dosing.

The cows were milked twice daily and composite samples of the two milkings were taken for days -1 to 6 and for days 28, 29, 31, 33, and 35. Separate morning and evening samples were taken for days 7, 14, and 21.

After sacrifice, samples of liver, blood, fat, muscle, and kidney were taken for analysis. The method of Kirkland et al (J. Ag. and Food Chem., 21, 171 [1973]) was used. This method has been successfully tried out by EPA. Adequate recoveries are reported for samples of milk, cream, skim milk, muscle, liver, fat, and kidney fortified at 0.01 to 0.05 ppm. A few fat samples had high recoveries, apparently due to an interfering co-eluting compound.

The highest levels found as a result of the three feeding levels are summarized below (no MBC [<0.01 ppm] was uncovered in any sample):

	Feeding Level					
	2 ppm		10 ppm		50 ppm	
Tissue	5-OH-MBC	4-OH-MBC	5-OH-MBC	4-OH-MBC	5-OH-MBC	4-OH-MBC
Muscle	<0.01	<0.05	<0.01	<0.05	<0.01	<0.05
Liver	<0.01	<0.05	<0.01	<0.05	0.01	0.05
Kidney	<0.01	<0.05	0.02	<0.05	0.06	<0.05
Fat	0.02	0.09	<0.01	<0.05	0.01	<0.05
Milk	0.01	<0.01	0.04	0.03	0.10	0.07

In addition the same analytical method was used to determine the level of MBC and its metabolites in the milk of cows that had been dosed with ^{14}C MBC. Nine milk samples were analyzed. No MBC, per se, was found by either the cold method or by counting activity. The levels of 5-OH-MBC and 4-OH-MBC were in general agreement: the level of 5-OH-MBC was 0.07 to 0.12 by the cold method and 0.09 to 0.13 by counting radioactivity; the levels of 4-OH-MBC were 0.04 to 0.08 by the cold method and 0.04 to 0.07 by counting radioactivity.

"Benomyl Livestock Feeding Study; Meat and Milk"

This study was completed in 1970 and submitted with PP#1F1010; it is discussed in our review of that petition (memo of 3/29/71, W. Boodee). Briefly, dairy cows were fed benomyl at levels of 2, 10, or 50 ppm in the diet for 32 days. No residues of benomyl or MBC were found in any tissues or in milk as a result of these feeding levels. The method of Kirkland et al, cited above, was used. Residues of 5-OH-MBC and 4-OH-MBC were detected milk and tissues as summarized in the following table:

	Feeding Level					
	2 ppm		10 ppm		50 ppm	
Tissue	5-OH-MBC	4-OH-MBC	5-OH-MBC	4-OH-MBC	5-OH-MBC	4-OH-MBC
Muscle	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Liver	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Kidney	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Fat	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Milk	<0.01	<0.01	0.01	<0.01	0.06	0.04

Thus residues as a result of feeding benomyl are somewhat lower than from feeding MBC. The differences are not great though and could be primarily a result of better application of the analytical method in the later (MBC) study.

Other Considerations

In addition to the above studies, the petitioner has also submitted an argument that the hypothetical diet used by RCB to calculate the maximum theoretical intake of benomyl is unreasonable. Dupont has consulted dairy scientists and arrived at what they consider to be reasonable dairy cow diets. However, none of these diets include the feed items, grape pomace and bean vine forage, with high benomyl tolerances. If Dupont wishes to establish that grape pomace and bean vine hay would not be used as diary feed items, they should submit written arguments from experts in diary science as well as from the grape and bean industry. Names addresses, and telephone numbers of those consulted should be included. It should be borne in mind that RCB is not as interested in what constitutes an ideal diet as it is interested in what the possibility is that grape pomace or bean vine forage could be used to feed dairy animals.

Conclusions and Recommendations

The submitted studies provide a basis for determining the level of benomyl expected in milk. In the absence of compelling data establishing that grape pomace and bean vine forage are not fed to dairy cows, RCB will recommend for a tolerance based on the following diet (first presented in E. Haeberer's memo of 8/15/84, in which protocols for benomyl and MBC metabolism and feeding studies are recommended):

Feed Item	% of Diet	Tolerance	Calculated Residue (ppm)
grape pomace	20	125	25
bean vine forage	37	50	18.5
grains	43	0.2	0.1
			43.6

The feeding studies show that a dietary intake of 50 ppm benomyl would result in a maximum residue in milk of 0.17 ppm; a dietary intake of 44 ppm would therefore result in residues of up to 0.15 ppm in milk. A milk tolerance of 0.2 ppm would be appropriate and should be proposed.

The question of a plateau level for bound residues in cow liver remains to be resolved.

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